Oxidative Phosphorylation & Mitochondrial Metabolism

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Alkylrhodamines as Cationic Protonophores

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Mild uncoupling of mitochondria assumes that partial decrease in membrane potential is beneficial for cells especially under some pathological conditions, suggesting that uncouplers are good candidates for therapeutic uses. The majority of known protonophoric uncouplers are weak acids capable to permeate across membranes in neutral and anionic forms. In the present study, protonophoric activity of a series of derivatives of cationic rhodamine 19 (including dodecylrhodamine C12R1 and its conjugate with plastoquinone SkQR1) but not of similar derivatives of rhodamine B was revealed by using a variety of assays. In planar bilayer lipid membranes, diffusion potential of hydrogen ions was formed in the presence of C12R1 and SkQR1. These compounds induced pH equilibration in liposomes loaded with the pH probe pyranine similarly to the well-known uncoupler FCCP. C12R1 and SkQR1 also stimulated respiration of rat liver mitochondria and decreased their membrane potential as measured by DiS-C3-5. The protonophoric activity of alkylrhodamines strongly depended on the number of carbon atoms in their alkyl chain (*n*): it was maximal at *n* about 10-12 and substantially decreased at *n* = 16. We surmise that the proton transport involves translocation of neutral and protonated cationic forms of C12R1 across a membrane. Based on the fact that SkQR1 accumulates in mitochondria of mammalian cells and protects them from oxidative stress (Bakeeva et al. 2008, Biochemistry Moscow, 73:1288-1299), it can be proposed that the protonophore-induced uncoupling could be involved in the protective effect of SkQR1 along with the direct antioxidant effect of plastoquinone.

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Structural and Functional Effects of C-Terminal Truncations in Cytochrome c Oxidase Subunit III from *Rhodobacter Sphaeroides*

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Cytochrome *c* oxidase (COX) catalyzes the oxidation of ferrocytochrome c and reduction of oxygen to water while pumping protons across the inner mitochondrial or bacterial membrane. In this work, two *R. sphaeroides* COX subunit III (SIII) truncation mutations which mimic those observed in human mitohcondria were characterized. One truncation created was after the third helix of SIII (Δ114 SIII), while the other was after the sixth helix (Δ238 SIII). Isolated and purified Δ114 SIII and the Δ228 SIII retained at least 70% and 30%, respectively of its SIII content. MALDI-TOF showed that Δ114 SIII and I/II COX (where SIII was genetically deleted) had altered *in vivo* processing of subunits II and IV. Electron transfer assays showed that Δ114 SIII and Δ238 SIII were inhibited by 50% and 70%, compared to control COX. The pH dependencies of the electron transfer rate in both mutations and I/II COX were similar (pKa= 7.4), as compared to wild-type (pKa= 8.7). Both mutations underwent turnover induced inactivation; however, when Δ114 SIII was supplemented with 1 mg/ml asolectin in the assay buffer, the catalytic lifetime of increased two fold when compared to I/II COX. Δ114 SIII and Δ238 SIII exhibited proton-pumping stoichiometries of 0.40 and 0.32, as compared to 0.76 wild-type COX and 0.38 for I-II COX. These results indicate that the five C-terminal helices in SIII play a critical role in the processing of subunits II and IV and in the assembly of COX. Also, structural lipids within the v-shaped cleft of SIII are necessary for providing protection against turnover induced inactivation, and that perturbation in the structure of SIII results in a lower efficiency of vectorial proton pumping by modifying the conformation of subunit I.

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The Thioredoxin 2 system Controls H2O2 Emission Flux from Mitochondria

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Respiring mitochondria continually produce hydrogen peroxide (H2O2). When production exceeds scavenging capacity, increased H2O2 emission will occur, thus transforming potential signaling into overt oxidative stress and cellular dysfunction. Glutathione and thioredoxin (Trx) are two major systems providing reducing equivalents, yet their relative contribution in keeping proper mitochondrial redox balance is unclear. Here we hypothesize that under physiological forward electron transport (FET) mode Trx2 is a major ROS buffering system in mitochondria. Thioredoxin reductase 2 (TrxR2) reconverts oxidized Trx2 into its reduced, active form, in mitochondria. Utilizing the highly specific TrxR2 inhibitor Auronofin (AF) we measured H2O2 flux from isolated mitochondria and ventricular myocytes obtained from C57/BL6 mice or guinea pig hearts. In isolated mitochondria, H2O2 emission and the redox status of Trx2 were analyzed in parallel in the absence or presence of AF, under states 4 and 3 of respiration triggered by Glutamate/Malate (G/M, 5mM) and 1mM ADP, respectively. Mitochondria preincubation with AF resulted in 20-fold or 14-fold rise in H2O2 levels with K0.5 of 4.8 ± 0.5 or 5.8 ± 0.6 nM in states 4 or 3 respiration, respectively, In energized and actively phosphorylating mitochondria we measured a $56 \pm 8\%$ rise in reduced Trx2 abundance, equivalent to an increase in reductive potential from -330 ± 4 to -348 ± 6 mV, which could be fully prevented by preincubation with 100nM AF. Importantly, AF had no effect on mitochondrial membrane potential, NADH, or GSH levels, reinforcing the high selectivity of AF while demonstrating the major H2O2 scavenging role played by the Trx2 system. In keeping with the results observed in isolated mitochondria, administering AF to cardiomyocytes provoked a rise in total cellular O2.- and H2O2 levels, resulting in reduced cell contractility. Our data indicate that the Trx2 system controls mitochondrial redox balance, H2O2 emission, and ultimately cardiomyocyte function.

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Mitochondrial Respiratory Chain Alternative Components Activity During Different Growth Phases in Yarrowia Lipolytica

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Mitochondrial respiratory chain alternative components activity during different growth phases in *Yarrowia lipolytica*.

In *Yarrowia lipolytica* mitochondria, the respiratory chain is constituted by the classic complexes plus an external NADH dehydrogenase (NDH2e) and an alternative oxidase (AOX). This results in a branched electron flux converging and diverging at the ubiquinone oxido-reduction. The oxidation of external NADH and reduction of oxygen via alternative components (NDH2e-ubiquinone-AOX) does not contribute to the proton-motive force and must be regulated depending on the cell energy requirements. Since more energy production is needed in the logarithmic growth phase, the futile oxidation of NADH may be prevented by electron channelling if either NDH2e or AOX bind to the classic complexes. It was observed that in the logarithmic growth phase NDH2e-derived electrons were specifically directed to the cytochrome complexes. In addition, the presence of respiratory supercomplexes plus the interaction of NDH2e with these complexes were evaluated using different techniques. NDH2e was detected in association with the cytochromic pathway. In contrast, in the stationary growth phase NDH2e-derived electrons were oxidised both, by the alternative and by the cytochromic pathway. This is in agreement with a higher NADH dehydrogenase activity detected in the stationary growth phase in combination with the lower activity of the cytochromic complexes and enhanced AOX. In stationary growth phase NDH2e no longer co-migrates with the cytochrome *c* oxidase when separated by ion exchange chromatography. The redirection of electrons from the cytochromic to the alternative pathway during the stationary growth phase is probably due to a combination of several factors: i) less amount of respiratory complex IV, to which NDH2e is believed to associate with; ii) higher transmembrane potential reached in the stationary growth phase and; iii) overexpression of NDH2e.

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Regulation of Mitochondrial Permeability Transition by ADP

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Mitochondrial ADP serves at least two important functions: provides a substrate for ATP generation and inhibits the mitochondrial permeability transition pore (mPTP). The mechanism for the inhibitory effect of ADP on mPTP remains unclear. Moreover, the contribution of this ADP-dependent regulation of mPTP in protection from mitochondrial injury has yet to be elucidated. We investigated the inhibitory effects of ADP on mPTP in mitochondria isolated from mouse hearts by measuring mitochondrial Ca^{2+} uptake capacity. Mitochondria isolated from control mice were able to sequester Ca^{2+} from up to 10 pulses of 40 μM Ca^{2+} (389 ± 45 μM, n=7) before the occurrence of a sudden and irreversible rise of Ca^{2+} in the incubating solution, indicative of mPTP opening. The presence of 500 μM ADP shifted the Ca^{2+} uptake capacity to 867 ± 89 μM (n=6), similar to that of cyclosporine A treatment (900 ± 139 μM, n=4) or cyclophilin D knockout mice (1300 ± 212 μM, n=4). Interestingly, ADP further increased the Ca^{2+} uptake capacity in cyclophilin D knockout mice (2075 ± 284 μM, n=4). 1 mM ATP and 10 mM creatine mimicked the effects of ADP (800 ± 124 μM, n=4). Also, oligomycin augmented ADP efficacy. Addition of 10 μM of atractyloside abolished the ADP-mediated increase in Ca^{2+} uptake capacity. The effect of Ca^{2+} on mPTP opening may be related to the production of ROS. The increase in ROS caused by Ca^{2+} addition is reversed by ADP, cyclosporine A, and in cyclophilin D knockout mice. In summary, we